

Some Studies and Comments on Hepatic and Extrahepatic Microsomal Toxication-Detoxication Systems.

A Limited Discussion of Some of the Heterogeneities of These Systems and of Their Responses to Stimulation of Enzyme "Induction"

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Introduction

Much of what will be discussed in this paper can be referred to as drug metabolism, or drug-metabolizing systems and some factors which affect these systems, for example by causing enzyme induction. If such terms as "drug metabolism" and "enzyme induction" are not further defined and explained, many people will still know what I am talking about and referring to. However, this sloppy language may needlessly restrict the interest and importance of some of the research going on in this field. Attempts to overcome this language-semantics barrier have been partly successful and need to be refined and extended. Some points needing emphasis in my opinion are: (a) drug metabolizing systems have a number of substrates that are not drugs. Some of these are "normal" substrates such as steroids, fatty acids, vitamins, and hormones. Other substrates include chemicals that are present in our food (such as preservatives, coloring agents, food additives for taste, odor control

and other purposes), or water (such as chemicals used in purifying the water or pollutants that cannot be removed), or air (odors, perfumes, and particulates—some added deliberately, some we can't or don't remove) and which we ingest in the process of living and working in an environment increasingly filled with effectors of these enzyme systems. To replace "drug metabolizing enzymes" with "xenobiotic or foreign chemical metabolizing systems" recognizes part of the problem—some of the substrates of these enzymes are not drugs; (b) drug or xenobiotic metabolizing systems can cause activation *and* inactivation of chemicals—sometimes even in sequence with or on the same substrate. Thus, changes in activity of the drug or xenobiotic metabolizing systems can either increase or decrease the toxicity of chemicals serving as substrates of these systems. Such changes can be specific for one substrate or apply to several substrates and at the same time may make one substrate more toxic and another less so. What often determines toxicity and actions of a chemical is the balance between metabolism to toxic vs. less-toxic metabolites. This balance may be shifted by increasing any or all of these metabolisms or by increasing some more than others, as well as by the

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simpler examples of increasing one kind (toxication) of reaction and decreasing another (detoxication) or *vice versa*. Thus, two points come out—drug or xenobiotic metabolizing systems are not just “detoxication mechanisms” and increases or induction of these systems can lead at the same or different times to intoxication as well as detoxication relative to the “before induction” stage; (c) toxication-detoxication systems include many reactions that are not oxidative in nature, are not cytochrome P450 requiring, are not restricted to the liver, and can occur elsewhere than in the microsomes. Therefore, to talk only about hepatic microsomal drug oxidations and further to consider only those which involve cytochrome P450 is to talk about a very small (but vigorously and intensely studied) part of the body’s systems for metabolizing endogenous and exogenous chemicals.

One cannot really deal with these three problem areas merely by recycling the nomenclature. Yet our use of convenient but inexact items can, by repetition, cause some dulling of our appreciation of the problems we are making for ourselves and colleagues in other disciplines who need to inform us and *vice versa* about common research efforts with these systems. People who work on “drug metabolizing enzyme systems” will tend to use drugs as substrates and think of the systems primarily in terms of their roles in therapeutics. Scientists who study “xenobiotic metabolizing systems” will think largely in terms of non-drug substrates and perhaps concentrate on the role of these systems and their interactions with model substrates, industrial chemicals or environmental pollutants. Research on “steroid or fatty acid metabolizing systems” will tend to focus on the role of these enzymes in regulating homonal functions and body homeostasis. Studies on “vitamin and non-steroid hormone metabolisms” will often concern itself with nutritional balances, intermediary metabolism and similar systems. The point being that words do make a difference, labels do matter, and we ignore this human weakness with some costs attached. One can

throw up his hands in despair at this point since alternatives to “drug metabolizing enzymes” are not easy to sell to anyone, let alone think up. But the lack of viable alternatives has led to a lot of “clubs” who do not communicate well with each other and the whole subject suffers. I will now close this part of the introduction by choosing my label for these systems: *toxication-detoxication systems*, only wishing I could somehow also add: . . . that may use as substrates drugs, foreign chemicals, or endogenous lipid-class members such as steroids, fatty acids, and vitamins, or endogenous non-lipids like thyroxine, and certain amines (even epinephrine or precursors), and may even be systems that neither toxify nor detoxify, but rather only metabolize these various substrates, and whose metabolism can be characterized as oxidative and non-oxidative, synthetic and hydrolytic, involving many organs besides liver, and including a lot of non-cytochrome P450 dependent systems.

Having spent so much time on broadening perspectives, I must now hasten to assure the reader that I cannot possibly deal with all these matters in this paper. Indeed the main subjects covered will be few in number and terribly selective since I prefer to write about my own work and not that of others. This will not be a general review of drug or xenobiotic metabolism, since I think plenty of these already exist. Instead, I wish to write about three research projects that have been under study in my laboratory over the last few years, and which I feel are of relevance to the interface between “drug” metabolism and environmental health science as disciplines having identity problems that are not dissimilar. These research projects I wish to concentrate on are: (a) extrahepatic toxication-detoxication systems; (b) age-dependent changes in toxication-detoxication systems in lung vs. liver, and (c) species-strain-individual differences in “induction” of hepatic toxication-detoxication systems by the pollutants benzpyrene and DDT.

If the reader wishes a general review of “drug metabolism,” or even specifically drug

metabolism and its role in environmental health, he should read elsewhere. Several very good reviews of the subject of "drug metabolism" and selected aspects of the systems in liver, especially those requiring cytochrome P450 have appeared quite recently. Some of these reviews are in book form; others are in journals or monographs. Many conferences are being held on the subject and the conference proceedings often appear very shortly thereafter. I cannot begin to list all such "reviews" that have been published even in the last 3 or 4 years. Inevitably, I would leave a key reference out, and probably that of a good friend.

Having thus dusted off a few old grievances and given you some idea of what I will say and will *not* even attempt to do, I can now turn to my subject with a somewhat less guilty conscience.

Extrahepatic Toxication-Detoxication Systems

The major research efforts in the field of "drug-metabolizing enzymes" have been concerned with systems in the liver. It is true that the liver of most animal species studied is especially rich in enzyme systems that metabolize chemicals of all kinds. It is also true that most workers have been aware that other body tissues had the ability to metabolize chemicals, but the reasons for the relative lack of studies of these other organs are numerous and probably include: (a) ease of handling the tissue - isolating it for study *in vivo* and *in vitro* as in perfused organ systems, slices, homogenates, and subcellular fractions - few tissues can match liver in manipulability for experimental study at all these levels of organization; (b) heterogeneity of structure - irregularities of blood flow or supply, lobular architecture or similar organ area differences, number of different cell types - the liver is almost ideally homogeneous, having relatively uniform lobules, and only two or three major cell types which can now be separated from each other for further study if desired; (c) ability to detect and quantify the metabolizing systems of interest - problems in quan-

tifying low levels of component or enzyme activity especially in the presence of high blanks due to things like blood - the liver has high levels of most toxication-detoxication systems and contaminants or inhibitors of assays can often be removed easily or at least lowered by perfusion, washing, etc.; (d) stability of systems - problems in preparing components or enzyme systems for assay before they are lost by cytolysis or autolysis, problems in storage of systems (freezing), problems in long-time incubation especially with low enzyme activities - the liver decomposes slowly, stores well, and is stable for most incubation or assay periods especially since content or enzyme activity is so high; (e) quantity of tissue available vs. quantity of tissue needed for assay, statistics, etc. - high activity and plenty of homogeneous tissue makes liver almost ideal in experiments calling for repetitive sampling or biopsy, estimation of individual variability, purification of the enzyme systems, etc.

Early studies in "drug metabolism" did occasionally include comparisons of liver with other tissues, at least in the common laboratory animals. From such studies it was learned that most organs of excretion or exchange were likely to have at least some capacity to metabolize chemicals. However, it was also obvious from this early work that in most cases, the variety of toxication-detoxication systems was certainly much less in extrahepatic tissues (than in liver), their activities (or levels) were often barely quantifiable and likely to be highly variable in "amount" from one species to another and even one animal to another - seemingly much more so than with liver. From time to time publications have appeared dealing with the metabolism of compound "X" by some extrahepatic tissue. Few if any studies have attempted to characterize the "drug metabolizing systems" of extrahepatic tissues in anything like the way done with liver. Among the problems that seemed to be rather consistently overlooked were: (a) Does the perfused whole organ metabolize the drug or remove it from blood by bind-

ing, storage or in some cases excretion?; (b) Do slices of the organ metabolize the drug (or store it, or excrete it)?; (c) What might be the activity of drug-metabolizing enzymes in homogenates and subcellular fractions (mitochondria, nuclei, lysosomes, microsomes, soluble fraction) and how does this compare (per gram organ) with activity in slices or in whole perfused organ?; (d) Have these subfractions of the homogenate been characterized by electron microscopy and biochemical markers?; (e) How great a problem is created by having many different cell types in the organ - which of these cell types are active in drug metabolism?; (f) Have a variety of substrates been used, and have enzyme assays been optimized with respect to protein dependence, substrate concentration, pH and cofactor requirements, ionic strength, etc.?; (g) What kinds of endogenous inhibitors or activators may be present *in vivo* vs. *in vitro* and can these inhibitors or activators be removed and isolated for study?; (h) What are the species, strain, sex, and individual variations seen in whether these systems are present (or absent) and what is the variation in level of activity of these extrahepatic systems *in vivo* and *in vitro*? What is the role of genetics vs. environment in the control of these variances?

Among tissues of obvious relevance to problems of environmental pharmacology and toxicology are those at the portals of entry or contact between ourselves and our environment. Metabolism of chemicals at such entrances or contact spots could be most important to the action of these chemicals - leading to activation or fixation of the chemical at its site of metabolism, or detoxication and excretion of the chemical. Among the first organs that we sought to characterize with respect to toxication-detoxication systems, and to compare these with the analogous liver systems, was the lung. We were aware of the fact that lung had been shown to have some ability to metabolize chemicals and that animals seemed to differ widely with respect to levels of toxication-detoxication systems in

lung. We chose to study metabolizing systems in rabbit lung since this animal has high levels of several "drug metabolizing enzymes" in its lung, and the mass of tissue per animal made it more likely that we could study systems in individual animals (1). There was also a system to study drug metabolism in an isolated perfused lung (2).

Our first studies were concerned with characterizing various treatments of lung to obtain homogenates, and then with the isolation from such homogenates of representative fractions such as nuclei, mitochondria, and microsomes. We hoped to identify these subcellular fractions on the basis of marker enzyme activities, using markers that were regarded as highly specific for the analogous hepatic fractions.

A major problem arises just in getting a reasonably good homogenate of lung tissue. The compromise needed is between rupture of at least a significant fraction of the cells and extensive fragmentation of particulates like mitochondria. We obtained what we consider to be a reasonable approach to such a compromise by just mincing the tissue extensively with an ordinary meat grinder or tissue press, and then homogenizing in a teflon-glass Potter homogenizer. The homogenate resulting from such manipulation can then be subfractionated into nuclei, mitochondria, microsomes and soluble fractions, though the correspondence of these lung fractions with analogously prepared liver fractions may not be too exact. There seems to be much less cell disruption in the case of lung than liver and a greater degree of cross-contamination as seen both in electron micrographs of the fractions and in terms of marker enzymes. Especially interesting was the finding of a meshwork in the lung microsomal fractions; this mesh seemed to tie together several microsomal vesicles and ribosomal particles into a kind of clump. We believe the presence of such a meshwork will make study of lung microsomal subfractions especially difficult. Thus, liver microsomes can be subfractionated into several types rough-surfaced vs. smooth-surfaced (3-11) as well as further subdivisions of each of these

(fractions of rough-surfaced membranes; magnesium binding vs. non-binding types of smooth-surfaced membranes (12, 13)). These microsomal subfractions in liver can be relatively pure (by electron microscopy) and contain differing amounts of drug-metabolizing enzymes. Similar studies applied to the lung could be misleading unless some way is found to break up the meshwork found in the microsomal fractions. At present, we are studying the use of sonication to break up these meshworks and we find that sonication helps to increase the yield of microsomes per gram liver *and* lung and the percentage of a microsomal marker enzyme (benzphetamine demethylase) which is recovered in the microsomal fraction of liver *and* lung. For example, of the total benzphetamine demethylase activity in the unsonicated homogenate, we find 60-80% of this in the microsomal fractions prepared from liver homogenates but only 20-25% of it in the microsomes from lung homogenate. When the homogenates are sonicated even briefly (10 secs-30 secs), the recoveries of activity in microsomes are markedly increased at least in lung. Thus in liver, recoveries range from 80-100% of homogenate activity in microsomes while in lung from 50-75% of the homogenate activity is now present in microsomes (14). This suggests that sonication is a very useful adjunct to routine homogenizing for purposes of increasing recovery of microsomes and contents from tissue homogenates. The effects of sonication were especially marked with lung and we believe this may result from effects on the microsomal meshwork.

Our work with marker enzymes and substrates of the toxication-detoxication systems showed that lung and liver fractions were similar in many respects. Many of the "drug-metabolizing" systems were concentrated in lung microsomes just as they are in liver. Major differences between lung and liver seemed to be (a) glucose-6-phosphatase was not a good marker for lung microsomes; (b) glucuronyl transferase activity was not detectable in lung microsomes; (c) cytochrome b₅ and P450 contents of lung micro-

somes were much lower than liver microsomes; (d) many "drug metabolisms" were also much less active in lung than liver microsomes although some metabolisms were equally active in microsomes from the two tissues of the rabbit. These results are found in a series of publications from our laboratory (14-20).

A number of other research projects have been stimulated by these studies. For one thing the lung, unlike the liver is quite heterogeneous - having marked morphological differentiations (lobules, bronchi, alveoli) as well as a plethora of different cell types. These lung cell types are difficult to isolate in pure or homogeneous populations. When we homogenize whole lung we are sampling a kind of average of all cells in the lung at the time of death. Since we found that lung microsomes from such a heterogeneous sample did contain appreciable levels of toxication-detoxication systems, it became important to identify which cells had these systems and whether certain systems might be found in only a few or even one kind of cell. Several approaches to this problem are possible and we hope to use several of these: (a) histochemical - identify at least qualitatively and perhaps even semi-quantitatively where certain of the toxication-detoxication systems might be found by applying substrates whose metabolites can be fixed, stained and identified in tissue sections; (b) separation of cells by digestion of organ minces with collagenase hyaluronidase, etc., to liberate cells which may then be separated on the basis of size, charge, shape, etc., using gels, electrophoresis, centrifugation, etc.; (c) selective stimulation or inhibition of lung cell growth - certain factors, diseases, chemicals or stresses applied to the animal may kill off a major cell type or cause its proliferation to markedly change its proportion of the total. This might be combined with method (b) to give even better separations for study, providing the factor, disease, stress or chemical did not affect cells not stimulated or killed *and* did not cause losses of toxication-detoxication systems even in cells stimulated

to proliferate; (d) growth of cells in culture or use of lung tumors - this often results in favoring one cell type over another.

Even before these studies are done, one or two simple experiments can be tried - for example lung washouts (lavages) can give cell populations especially rich in alveolar macrophages. When we subjected such cells to analysis for toxication-detoxication systems we found little if any detectable (20). Thus at least one of the major cell types in lungs contributing to the lung microsomal fraction is *not* a storehouse or site of concentration of toxication-detoxication systems.

Age-Dependent Changes in Toxication-Detoxication Systems in Lung vs. Liver

The newborn of several animal species are relatively deficient in the hepatic microsomal "drug metabolizing" enzyme systems. Such a defect probably contributes to the relative sensitivity of the newborn (compared to adults) to the actions and toxicities of a number of chemicals and drugs that are detoxified by these systems. I have been interested in the age-related changes in toxication-detoxication systems for many years, with most of my studies dealing with the liver systems (21-27). Having found lung to contain appreciable amounts of some analogous metabolic systems (15-20), we were interested in how these lung systems compared with liver during the first month of life. We were also hoping to see whether different parts of some of the toxication-detoxication systems might develop at quite different rates in liver vs. lung, and to gain some more insight into whether the rate-limiting step for certain reactions might be different in liver vs. lung.

In the rabbit, we found that two general patterns of development of enzyme activity and cytochromes seemed to be present: (a) a slow gradual increase in activity and level during and beyond the first month of life - typical of most of the systems in the lung and (b) a gradual increase to an age of about 2 weeks and then a sudden jump to adult levels of activity - typical of most systems in the liver (14).

Another finding of interest was that drug metabolisms, cytochrome b_5 and P450 levels and activities of NADPH-cytochrome c and P450 reductases did not correlate well at the various ages in either lung or liver. It was also obvious that at least in the lung a major problem would arise in trying to equate the rate-limiting step of drug metabolism with the activity of NADPH-cytochrome c reductase. Various laboratories have tried to identify the key (rate-limiting) step in cytochrome P450-dependent drug metabolisms in liver microsomes. At the present time, many workers believe that for mixed function oxidases (MFO) in these liver microsomes, the most likely candidate for this key role is NADPH-cytochrome P450 reductase (P450 reductase) activity. Thus rates of MFO-catalyzed detoxication-reactions in liver microsomes do not correlate well with cytochrome P450 or b_5 levels, but do agree fairly well with P450 reductase levels. Even better correlation may be found if the P450 reductase activities are measured in the presence of substrates or other effectors of the systems, since P450 reductase activity is affected by substrates (29) and metal ions or ionic strength (30, 31).

At all ages studied, lung and liver microsomes had very similar but age-dependent levels of NADPH-cytochrome c reductase and where measurable, NADPH-cytochrome P450 reductase. Our preliminary studies on these enzyme activities and their response to added ions or substrates suggest that the responses of these enzymes are also similar in the two organs, at least in the adult rabbit. We have now studied several substrates of liver vs. lung microsomal toxication-detoxication systems. Two groupings can be made: (a) substrates whose rate of metabolism per mg microsomal protein per unit time is the same or greater in lung than in liver microsomes (benzphetamine dealkylation, biphenyl hydroxylation, ethylmorphine demethylation, or p-chloro-N-methylaniline demethylation), and; (b) substrates or components whose metabolism or level per mg protein is much less in lung than in liver microsomes (benzpyrene

hydroxylase, aminopyrine demethylase, aniline hydroxylase, p-nitrobenzoic acid reductase, and the cytochromes b_5 and P450). If activity is expressed per nmole P450 per unit time then nearly all metabolisms in lung are equal to (group b) or many times greater (group a) than those in liver.

If NADPH-cytochrome c reductase is the same enzyme as NADPH-cytochrome P450 reductase (32, 33), and if NADPH-cytochrome P450 reductase is the rate-limiting step for MFO's dependent on cytochrome P450, then it is not a simple matter to understand how the rate-limiting steps in lung and liver can be at the same level while substrate metabolisms such as benzpyrene hydroxylase, aminopyrine demethylase, and aniline hydroxylase differ by factors as great as five-fold in lung vs. liver. The result is not qualitatively changed whether activity is expressed per mg protein or per nmole of P450; the discrepancy is just as large. But now the question changes: How can the rate-limiting step in lung be so much greater than in liver when the metabolism of several chemicals (aminopyrine, aniline, benzpyrene) occurs at the same rate in the two organs?

Species Differences in Induction of Hepatic Toxication-Detoxication Systems by the Pollutants, Benzpyrene and DDT

The hepatic "drug-metabolizing enzyme systems" can be stimulated by a variety of chemicals when these are ingested by the animal. The stimulation usually takes several hours or days, and seems to involve synthesis of more of these systems. Chemicals which cause this hepatic enzyme "induction" can be divided into several classes on the basis of (a) how soon the increase can first be detected; (b) whether several microsomal systems or components are increased or only a few; (c) whether the increase is accompanied by a proliferation of the parenchymal cell (smooth) endoplasmic reticulum; (d) whether cytochrome P450 increases or a new cytochrome (P_1 450, P448) is formed; (e) whether and how much substrate-difference spectra (produced by adding

chemicals to microsomal suspensions in a spectrophotometer cuvette) are affected, and (f) effects on the kinetic constants of the drug or chemical metabolisms.

Two classes of hepatic drug-metabolizing enzyme inducers are well-studied, and typical members of these classes are benzpyrene or 3-methylcholanthrene vs. phenobarbital or DDT. The enzyme induction by benzpyrene can be detected between 6 and 12 hours after animals are treated with benzpyrene, whereas stimulation by DDT is detectable only much later; benzpyrene stimulates only a few enzyme systems while DDT causes increase in most of the toxication-detoxication systems in liver microsomes; benzpyrene does not cause increase in parenchymal cell smooth endoplasmic reticulum, whereas DDT usually induces a massive proliferation of this system; benzpyrene induces the formation of a spectrally different P450 (P_1 450, P448) that can be purified and shown to behave quite differently from normal P450, whereas DDT appears to promote the formation only of more P450; substrate difference spectra after benzpyrene seem to be increased only for those substrates whose metabolism is increased and most other substrate spectra are decreased or qualitatively changed, whereas DDT seems to cause only increases in spectra if any change occurs at all; benzpyrene seems to decrease K_m for those substrates whose V_{max} is increased, whereas DDT affects only V_{max} (per mg microsomal protein). In reality, DDT probably does not affect even V_{max} , since if expressed per nmole P450, there is little change in this "constant" as would be expected if only more, but not different enzyme is being produced.

Species and strain differences in response to inducers of hepatic microsomal drug metabolism have been described, but are not widely recognized. Such variability in response can be very large quantitatively, at which point it becomes almost qualitative (one species responding so poorly that for practical purposes there has been no induction). Such variability can be important in explaining different chemical toxicities

especially in tests of chronic toxicity, and in understanding interactions of chemicals or chemicals-drugs-environmental stresses, etc. Induction of toxication-detoxication systems can markedly shift such things as ED_{50} or LD_{50} or even qualitatively change chemical actions or toxicities.

The basis for these variations in response to inducers of the hepatic enzyme systems has been presumed to be both genetic and environmental, and both have now been described, though not in great detail. Whether and how much induction of the hepatic systems occurs with any given inducer does indeed vary with diet (starvation vs. diets with abnormal fat content, protein content, or vitamin content), age of the animal, time of day (or light-eating cycles), and presence or absence of other effectors of these systems (antibiotics, antimetabolites, pesticides, disease, stress), and these factors can be lumped together (with many others I have not listed) under the general class of environmental influences. Genetic control of enzyme induction can be seen in variations in response to inducers between sexes (in rats) as well as between individuals, strains, and species of animals.

Several years ago, we showed that strains of rabbits differed in response of their hepatic microsomal enzymes to phenobarbital administration to the rabbit (34). The differences seen involved not only the generality of whether a given strain seemed to respond to phenobarbital or not and how much, but whether a given hepatic drug metabolism responded in all or only a few rabbit strains. Among the six strains of rabbits, the California and English strains seemed to be generally less responsive to phenobarbital than the others, while Jack rabbits seemed to be more responsive to phenobarbital ("responsive" defined as total number of drug metabolisms significantly increased by phenobarbital compared with total number of drug metabolisms studied). As far as pathways were concerned, only one was induced in all strains (hexobarbital oxidase) while two were not increased in any strain (amphetamine deamination and chlor-

promazine sulfoxidation). Other pathways were increased in some strains, but not others. Some of these differences may have been environmentally induced rather than determined by genetics, since the wild rabbits (cottontails, jack rabbits) obviously could have had quite different diets, etc., both before and after delivery to our laboratory (wild rabbits in captivity do not eat, are highly stressed, etc.).

In later work, we discovered an apparent difference in response of rats vs. mice to induction of hepatic toxication-detoxication systems by benzpyrene and DDT (35-41). Thus, at doses and schedules of dosing and at times after last dose of the inducer that clearly established hepatic microsomal enzyme induction by DDT or benzpyrene in rats and other species including monkeys (42), the mouse did *not* show such induction or showed it only marginally (36). A detailed study of the mouse liver systems failed to uncover any evidence for enzyme induction by benzpyrene (38, 39), although we were able to show some enzyme induction by the related, but more potent polycyclic hydrocarbon 3-methylcholanthrene. Others have reported that mice are less likely to respond to hepatic microsomal enzyme inducers of the polycyclic hydrocarbon class than are other species, especially rats (43). Most of these other findings were made using 3-methylcholanthrene as the inducer, not benzpyrene, and the accumulated evidence began to suggest that we were dealing with a quantitative, not a qualitative difference - mice responded poorly to an inducer that worked well in rats.

A very similar picture emerged with DDT. DDT did not affect most mouse liver microsomal systems enough for the changes to be measured, whereas the related inducer, chlordane, could cause quantifiable responses in mice (35, 36). Again the accumulating evidence suggested a quantitative, not qualitative, difference in mouse vs. rat response to the hepatic enzyme inducers, DDT and benzpyrene.

However, work done in Dr. Nebert's lab (44, 45) suggested that mouse strains may

differ qualitatively in their response to induction of benzpyrene hydroxylase by polycyclic hydrocarbons. These workers have shown that inducibility is strain specific and gene dependent as a simple autosomal dominant trait. Thus the C57BL/6N mouse responded to inducers of benzpyrene hydroxylase, while the DBA/2N mouse did not. A most interesting result of these experiments was that the "resistant" mouse (DBA/2N) was able to respond to *other* classes of inducers such as phenobarbital. Also that resistance of benzpyrene hydroxylase to induction applied to extrahepatic tissues as well as liver. Other polycyclic hydrocarbons (than 3-MC) were effective or non-effective in the same way as 3-MC. Thus, none of these worked in any tissue of the DBA/2N mouse, though phenobarbital could stimulate hepatic drug metabolisms in this mouse.

Our most recent work with mouse liver responses to benzpyrene and DDT has used the same strain of mouse that we started with at Iowa, i.e., mice which did not respond to benzpyrene, but did respond to 3-methylcholanthrene and very slightly to DDT. In these latest studies we have greatly increased the dose of inducer used and shortened the period between last dose of inducer and the time of enzyme assay. We have now been able to show that this strain of resistant mouse (Swiss-Webster) can respond to benzpyrene and DDT as inducers of hepatic microsomal enzymes and components, but that the induction effect is very much less and much shorter in duration than with rats (40, 41). A finding of interest to us was that even in mice that showed measurable enzyme response to benzpyrene treatment, there was still no shift in the cytochrome P450-CO spectra such as occurs in other species (due to formation of P₁450 or P448). Nebert and coworkers (44, 45) have shown that mice responding to 3-methylcholanthrene treatment (the C57BL strain) showed a shift from cytochrome P450 to P448, while mice that didn't respond (by enzyme induction) showed no P450 to P448 transformation. Our experi-

ments (41) would appear to show that enzyme induction by benzpyrene in the Swiss Webster mouse does *not* require a synthesis of P448 as is believed necessary by most people in the field.

One other point may perhaps need emphasis before leaving this section. Although we attempted to use the same strain of mice in all our studies on DDT and benzpyrene as inducers in mice vs. rats, we may not have been able to do so. In the 7 years between our first reported experiments (35) and our latest studies (40, 41), it is unlikely that the Swiss Webster strain of mouse remained genetically unchanged. Thus, we may well have started with a mouse genetically unable to respond to either DDT or benzpyrene, and ended up with a mouse able to respond at least to high doses of these inducers. We hope to continue our studies on genetic controls of response to enzyme induction by benzpyrene or DDT in the same mouse strains used by Nebert and coworkers (44, 45).

Conclusion

This paper has considered only a few of the many sources of heterogeneity in microsomal toxication-detoxication systems and in the response of these systems to effectors such as enzyme inducers. It should be obvious that this heterogeneity has many bases including both genetic and environmental factors. Few of these have been sorted out or studied in any detail, and as we find more of these, it will be increasingly difficult to isolate only one such effector for a detailed study. Some of the newer technics may help us to simplify our experiments so as to more clearly identify and study single influencing factors - e.g. - *in vitro* culture technics to study enzyme induction on isolated homogeneous cell populations. It is unlikely that such simplified systems can eliminate all multifactorial interactions, but the approach will be useful regardless.

More important, in my opinion, is a wider recognition of the heterogeneity of these systems and their responses. Such recog-

nition could hopefully discourage the generalizations for which overeager problem-solvers always grasp. It is obvious from literally thousands of studies that toxication-detoxication systems are important determinants of the action and toxicity of a wide variety of chemicals, drugs, pollutants, etc. However, it is also becoming obvious that whether and when these systems play key roles in determining chemical effects, how they fluctuate and from what causes, can very much be almost whimsical in terms of the fragmentary knowledge we now possess. Better understanding will hopefully generate some more general principles than we now possess. My purpose in writing this paper was to emphasize how few generalities we now have and some of the areas that we have chosen to try to understand better.

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